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DOI: https://doi.org/10.1093/jpe/rtw096

Journal of
Plant Ecology

VOLUME 10, NUMBER 1,
PAGES 158–169

FEBRUARY 2017

doi:10.1093/jpe/rtw096

available online at
www.jpe.oxfordjournals.org

Interspecific and intraspecific variation in specific root length drives aboveground biodiversity effects in young experimental forest stands

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Abstract

Aims

Although the net biodiversity effect (NE) can be statistically partitioned into complementarity and selection effects (CE and SE), there are different underlying mechanisms that can cause a certain partitioning. Our objective was to assess the role of resource partitioning and species interactions as two important mechanisms that can bring about CEs by interspecific and intraspecific trait variation.

Methods

We measured tree height of 2493 living individuals in 57 plots and specific root length (SRL) on first-order roots of 368 of these individuals across different species richness levels (1, 2, 4, 8 species) in a large-scale forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China) established in 2009. We describe the effects of resource partitioning between species by a fixed component of interspecific functional diversity (RaoQ) and further effects of species interactions by variable components of interspecific and intraspecific functional diversity (community weighted trait similarity and trait dissimilarity, CWS and CWD). Finally, we examined the relationships between biodiversity effects on stand-level tree height and functional diversity (RaoQ, CWS and CWD) in SRL using linear regression and assessed the relative importance of these three components of functional diversity in explaining the diversity effects.

Important Findings

Our results show that species richness significantly affected SRL in five and tree height in ten out of 16 species. A positive NE was generally brought about by a positive CE on stand-level tree height and related to high values of RaoQ and CWS in SRL. A positive CE was related to high values of all three components of root functional diversity (RaoQ, CWS and CWD). Our study suggests that both resource partitioning and species interactions are the underlying mechanisms of biodiversity effects on stand-level tree growth in subtropical forest.

Keywords: aboveground complementarity effects, belowground resource partitioning, biodiversity and ecosystem functioning, species interactions, tree height

Received: 25 November 2015, Revised: 26 August 2016, Accepted: 18 September 2016

INTRODUCTION

The rapid loss of species due to human activities and its implications for ecosystem functioning, services and human well-being have prompted biodiversity research to grow into one of the most active fields in ecological research during the last decades (Cardinale 2012; de Mazancourt *et al.* 2013). A series of reviews and meta-analyses about effects of biodiversity on ecosystem functioning (BEF) based on a broad array of studies from terrestrial, marine and freshwater ecosystems (Balvanera *et al.* 2006, Hooper *et al.* 2012, Stachowicz *et al.* 2007) show that many ecosystem functions are significantly influenced by different aspects of biodiversity. In particular, various experimental and observational studies have reported a higher mean aboveground biomass production in mixtures than in monocultures, which is referred to as over-yielding. Mathematically, this net biodiversity effect (NE) can be partitioned into a selection effect (SE) and a complementarity effect (CE). Positive SEs occur if a high-diversity plant community assembled at random from a pool of species has a higher chance of containing and becoming dominated by the species that achieves the highest biomass when growing alone (Loreau and Hector 2001). Therefore, SE is a species-specific impact on biomass production that occurs if the most productive species is more likely to be included in the mixture (Cardinale *et al.* 2007). Alternatively, positive CEs occur if two or more species can achieve relatively higher productivity in the mixture than in monocultures of the same component species.

There are different underlying mechanisms that can cause positive CEs. A positive CE can be caused by (i) niche differentiation if coexisting species occupy different ecological niches, which results in more complete or efficient resource use (Brassard *et al.* 2011; Loreau and Hector 2001; Marquard *et al.* 2009; Spehn *et al.* 2005), and by (ii) facilitation if the presence of one species benefits other species by improving their growing conditions or other types of positive species interactions such as mutualism (Golubski and Abrams 2011). Therefore, mechanisms of biodiversity effects on ecosystem functioning may arise from three aspects: the biological identity and differences among species (a species-specific impact); niche differentiation (i.e. resource partitioning across a spatial or temporal scale) and species interactions (i.e. facilitation or competition among plant species, interactions among plants, animals and microbes).

Although Loreau and Hector (2001) provided a powerful mathematical method for *a posteriori* partitioning between SE and CE in BEF experiments, it does not allow to distinguish between effects of resource partitioning and other sorts of species interactions. Firstly, species can be complementary in resource use due to either trait differences or different resource niches on a chemical, spatial and temporal scale (McKane *et al.* 2002; Chi *et al.* 2017). Secondly, species can facilitate each other by improving habitat conditions such as increasing nutrient availability, e.g. by elevated soil nitrogen (N) availability by N-fixing legumes (Temperton *et al.* 2007). Moreover, it should be noted that CEs do not solely implicate positive species interactions. Negative interactions between plant species (negative CEs) can also occur if physical or chemical interference results in a lower productivity in mixtures than expected from monocultures (Polley *et al.* 2003).

With the development of functional ecology in the last decades, there is a growing consensus that community trait composition, i.e. the distribution of trait values of species in a community, greatly influences ecosystem processes and services (Díaz *et al.* 2007; Garnier *et al.* 2004; Lavorel and Mazancourt 2013; Petchey and Gaston 2006; Li *et al.* 2017). Functional traits are defined as morpho-physio-phenological traits that impact the fitness of individual species indirectly *via* their effects on growth, reproduction and survival, the three components of individual performance (Violle *et al.* 2007). In general, a community's trait composition is the result of environmental or biotic filters, determining which individuals with specific attributes (or response traits) are able to survive and persist in a community (Keddy 1992). In addition, traits in a community play a functional role in the community (effect traits, Suding *et al.*, 2008). In BEF experiments with randomly assembled communities from a common species pool, the response aspect of traits plays a subordinate role, as the species are forced into coexistence. Thus, the whole range in trait compositions can be investigated without having to take into account that some trait compositions have been filtered out in community assembly processes. Nevertheless, competition between different species can be expected to differ with respect to their traits. Competition for resources is currently recognized as a large part of the filtering effect in plant communities (Kraft and Ackerly 2010; Shi *et al.* 2017). Most studies focusing on competition impacts on community structure have been based on a common assumption: species with similar ecological strategies, generally closely related phylogenetically, compete more intensely for resources than species with different strategies. Thus, species with similar strategies have similar ecological niches and are less likely to coexist (referred to as 'competition-relatedness' hypothesis; Cahill *et al.* 2008). According to this hypothesis, competitive interactions increase with increasing trait similarity in interacting plants (Kunstler *et al.* 2012). Thus, interspecific trait variation can increase resource partitioning but also affect species interactions and thus overall ecosystem functioning.

Species identity is not the only source of functional trait variation because both abiotic and biotic environment influence plant trait values of species (Fort *et al.* 2013). Recent studies have started to shed some light on the importance of intraspecific trait variation for community functional responses (Albert *et al.* 2012; Andersen *et al.* 2012; Hahn *et al.* 2017; Jung *et al.* 2014; Li *et al.* 2017a, Zheng *et al.* 2017). Kunstler *et al.* (2012) proposed to consider intraspecific variation of traits when analyzing the link between plant trait attributes and species interactions, particularly if this variation is linked to growth conditions, i.e. if it is due to plasticity within rather than genetic differences between species. Additionally, intraspecific trait variation may cause

plant-strategy differentiation within particular habitats and hence be responsible for niche differentiation, determining whether species coexist within a habitat or not (Chesson 2000; Zuppinger-Dingley *et al.* 2014). Therefore, functional traits can indicate how a species relates and responds to its environment, which offers a powerful approach to addressing ecological questions (McGill *et al.* 2006).

In summary, biodiversity effects on particular ecosystem functions, in the case of the present study tree height, can rely on the following mechanisms: (i) SEs caused by specific traits of highly productive species included in mixture, (ii) CEs brought about by resource partitioning due to a broadening of the trait spaces of a community by multiple species or (iii) CEs caused by facilitation due to trait variation of species as a response to different biotic conditions. If resource-uptake traits of individual species do not vary plastically between monocultures and mixtures, CEs brought about by resource partitioning between species in mixtures can be considered as due to a fixed component of interspecific functional diversity (e.g. as measured by RaoQ, Botta-Dukát, 2005), and thus can be derived from the trait values of species in monocultures and the abundance of species in mixtures. In addition, there may be a variable component of interspecific functional diversity. If trait values of species differ between monocultures and mixtures, it means species interactions may occur and these plastic changes of traits may also contribute to CE by species interactions. This variable component of CE can be related to the variable component of interspecific functional diversity derived from the difference in trait values between monocultures and mixtures, as quantified by community weighted trait similarity (CWS, for details see Methods). Furthermore, if trait values of species differ between monocultures and mixtures, there is also a variable component of intraspecific functional diversity. This variable component of CE, reflecting species interactions, can be assessed by community weighted trait dissimilarity (CWD, for details see Methods). Thus, a trait-based approach has the potential to describe resource partitioning and species interactions by interspecific and intraspecific trait variation, respectively. In this way, the contribution of resource partitioning and species interaction to CE can be quantified by functional traits. As we will use some abbreviations in the following, an abbreviation list is presented in Table 1.

To our knowledge, although SE and CE have been partitioned in BEF experiments for more than 10 years, little progress has been made in elucidating the underlying mechanisms (Loreau and de Mazancourt 2013). Moreover, because root traits are major drivers in nutrient uptake and water use, our understanding of how these ecosystem processes are shaped will be enhanced by a better understanding of the role of root traits of species growing in monocultures or mixtures (Mommer and Weemstra 2012). Root functional traits, which are supposed to be the main drivers of early-stage competitive interactions, have received little attention so far (Belcher *et al.* 1995). In this paper, we focus on a particular fine-root trait (the first-order specific root length; SRL) which is relevant for a plant's water and nutrient uptake (Mommer and Weemstra 2012) and is considered as an important functional trait for tree growth (Reich *et al.* 1998; Comas and Eissenstat 2004; Sun *et al.*, 2017; Wang *et al.* 2017). We studied this trait and tree growth in subtropical tree species in a forest biodiversity–ecosystem functioning experiment in Xingangshan, Jiangxi Province, China (BEF-China). We made use of 16 tree species planted at different species richness levels, which allowed us to assess the effect of resource partitioning and species interaction on the CE from interspecific and intraspecific trait variation between monocultures and mixtures. As a measure of ecosystem functioning related to primary productivity we used tree height assessed for all planted trees in a previous study of Li *et al.* (2014, 2017b). This study showed a greater investment in height growth at the expense of diameter growth, suggesting that light interception was the priority for tree sapling growth in our experiment. Moreover, we examined the relationships between biodiversity effects (NE, SE, CE) and measures of functional diversity based on interspecific and intraspecific trait variation and assessed the relative strength of these relationships. Specifically, we tested the following hypotheses.

Table 1: Abbreviation list of variables

Abbrevation	Full name	Functional diversity
NE	Net effect	/
CE	Complementarity effect	/
SE	Selection effect	/
RaoQ	Rao's quadratic entropy	A fixed component of interspecific functional diversity
CWS	Community weighted trait similarity	A variable component of interspecific functional diversity
CWD	Community weighted trait dissimilarity	A variable component of intraspecific functional diversity

The symbol "/" represent empty cell.

(i) Positive biodiversity effects (NE, SE, CE) on stand-level tree height are significantly related to belowground species differences in fine-root traits, in particular SRL.
(ii) The fixed component of interspecific functional diversity (i.e. RaoQ) drives the CE, indicating a contribution of resource partitioning to the biodiversity effect.
(iii) The variable components of interspecific and intraspecific functional diversity (i.e. CWS and CWD, respectively) also affect the CE, indicating a contribution of species interactions to the biodiversity effect.

MATERIALS AND METHODS

Study site

The BEF-China experiment (Bruelheide *et al.* 2014) is located near Xingangshan township, Dexing, Jiangxi Province (29°08′–29°11′N, 117°90′–117°93′E). The climate of this

region is typical of the subtropics, with mean annual temperature of 16.7°C and precipitation of 1821 mm. January and July are the coldest and hottest months with mean temperatures of 0.4°C and 34.2°C, respectively. The natural vegetation is characterized by subtropical forest with a mixture of evergreen and deciduous species. However, most forested areas in this region have undergone a dramatic conversion from mixed natural forests to commercial plantations of *Pinus massoniana* and *Cunninghamia lanceolata* (Bruelheide *et al.* 2014).

In this study, we focus on site A of the BEF-China project, because it was established earlier (2009) than site B (2010), and thus shows a more developed canopy. Site A encompasses a hilly area of 26.6 ha ranging in altitude from 105–275 m and in slope from 0 to 45 degrees. The soils are Cambisols and Cambisol derivatives, interspersed with Regosols on ridges and crests, and Anthrosols from colluvial deposits on foot slopes and valley floors(Li *et al.* 2014; Scholten *et al.* 2017).

Experimental design

A total of 271 plots, each with a projected area of 666.7 m^2 (25.8 × 25.8 m), were established during the period November 2008 to April 2009. Three different random extinction scenarios, one replicate of one of these scenarios and two non-random extinction scenarios were used to create a gradient of species richness levels. In total, seven richness levels of 0, 1, 2, 4, 8, 16 and 24 tree species were assigned to 15, 88, 68, 40, 26, 19 and 5 plots, respectively. In the 246 plots with a richness level ≥ 1, 400 (20 × 20) individual tree saplings were planted at equal projected distances of 1.29 m in March 2009, resulting in a total of 107 200 trees. Each tree is tagged with a unique numeric code. The species pool is composed of 24 native broad-leaved tree species. Further details on the general design and establishment of the BEF-China experiment are given in (Bruelheide *et al.* 2014).

Data collection

Field sampling was conducted from September to October 2013 in 60 plots of one random extinction series with two replicates of each species composition. These compositions followed a so-called broken-stick design (Bruelheide *et al.* 2014) with a pool of 16 species: *Castanea henryi* (*CaHe*), *Nyssa sinensis* (*NySi*), *Liquidambar formosana* (*LiFo*), *Sapindus saponaria* (*SaSa*), *Triadica sebifera* (*TrSe*), *Choerospondias axillaris* (*ChAx*), *Quercus serrata* (*QuSe*), *Castanopsis sclerophylla* (*CaSc*), *Cyclobalanopsis glauca* (*CyGl*), *Quercus fabri* (*QuFa*), *Schima superba* (*ScSu*), *Rhus chinensis* (*RhCh*), *Lithocarpus glaber* (*LiGl*), *Koelreuteria bipinnata* (*KoBi*), *Cyclobalanopsis myrsinifolia* (*CyMy*), *Castanopsis eyrei* (*CaEy*). In the broken-stick design, each species is present in one community composition at every richness level (1, 2, 4 or 8 species; we excluded plots with 16 and 24 species). We planned to randomly sample each species three times per plot, resulting in a planned total of 384 trees (4 richness levels × 16 species × 2 plots × 3 individuals per species per plot). Due to logistic reasons (high mortality in some plots) and incomplete neighborhoods of species in plots, only 368 trees were sampled. In total, we sampled 30 monocultures (with two monoculture plots for 14 species and one monoculture plot for two species), 16 two-species mixtures (eight species pairs each with two replicates), seven 4-species mixtures (three 4-species mixtures with two replicates and one (*LiGl-KoBi-CyMy-CaEy*) with one replicate) and four 8-species mixtures (two 8-species mixtures each with two replicates). For root sampling in mixtures, we excluded trees that had no direct interspecific neighbors.

At least two randomly selected intact parts of root systems (maximum diameter 2~5 mm) were sampled by tracking lateral roots of target trees through the surface soil (depth 0~15 cm) from every tree. The sampled parts of root systems were placed in a plastic bag and stored in a portable cooler for the transport from the field to the laboratory. The soil was carefully removed from the root systems with metal probes and the root systems placed in water and gently stirred to dislodge the soil from the roots. The root systems were then dissected into roots of according to branch order. The most distal roots were classified as first-order roots, the roots from which two first-order roots branched off were classified as second-order roots, and so on, continuing to fifth-order roots (Pregitzer *et al.* 2002). Roots were scanned with a scanner by root order class and analyzed with WinRHIZO (Regent Software, Canada) to measure root lengths. After scanning, the roots were oven-dried at 60°C for over 48 h until they had constant weight and then were weighed. SRL (m g^{-1}) was calculated as ratio of fresh root length to dry mass. In this study, we focus on the first-order SRL, because SRL was considered as an important functional trait for tree growth (Comas and Eissenstat 2004; Reich *et al.* 1998; Sun *et al.*, 2017) and the first-order SRL can be expected to be the most sensitive fine-root trait regarding nutrient and water uptake (Ostonen *et al.*, 2007). Moreover, we found that SRL decreased with increasing root order for all the 16 species and was tightly related to other root traits, thus SRL of first-order roots conveniently represented root traits in general and analyzing multiple root orders would have implied multiple hypothesis testing with dependent data (see online supplementary Appendix Fig. A1 and A2). The complete data can be obtained from the authors.

Growth measurements referred to all available individuals in the central part of the same plots (Li *et al.* 2014), which comprised the central 6 × 6 planting positions (i.e. 36 trees per plot) in the monocultures and 2-species mixtures and the central 12 × 12 planting positions (i.e. 144 trees per plot) in the 4- and 8-species mixtures. A total of 2493 living tree individuals were observed in these positions, including the 368 trees from which roots were sampled. For each tree individual, tree height was measured with a measuring pole as the length from stem base to the tip of the highest branch in September 2013 (the fifth year after planting). We took the total height of all planted trees (including surviving trees and dead trees assigned height = 0) in the plot as a measure of biomass production, and thus of ecosystem functioning, as we could not harvest trees in this experiment. Moreover, a previous study

found that there was a greater investment in height growth at the expense of diameter growth in the planted tree saplings and that growth in the BEF-China experiment was better reflected by increases in tree height than in stem diameter (Li *et al.* 2014; Scholten *et al.* 2017). The calculations of NE, CE and SE for tree height were carried out according to Loreau and Hector (2001). Thus, NE is equal to the sum of SE and CE.

Statistical analysis

The fixed and variable component of the CE can be described by interspecific and intraspecific functional diversity. The possible cases of differences in trait values between monocultures and mixtures are shown in Fig. 1. If the interaction between two species is neutral, the trait distances (the difference between functional trait values of species A and B) in the mixture should be the same as the one in the monocultures (Fig. 1B). If the interaction between two species is positive or negative, trait distances in the mixture should differ between monocultures and mixtures, leading either to trait convergence or divergence in the mixtures (Fig. 1C and D). If species do not differ in trait values between monocultures and mixtures (Fig. 1B), the component of CE (i.e. resource partitioning) would only be brought about by the fixed component of interspecific functional diversity. The fixed component of interspecific functional diversity was assessed by RaoQ (estimated by the FD package in R with the function dbFD; Laliberté and Legendre 2010). RaoQ expresses the mean trait distance between two randomly selected individuals (Botta-Dukát 2005). If species differ in trait values between monocultures and mixtures (Fig. 1C and D), a variable component of interspecific functional diversity contributes to CE (species interactions), which can be derived from the difference in trait distances between monocultures and mixture. According to the competition trait, similarity hypothesis competitive interaction strengths between species increase with decreasing niche distance, measured as absolute trait distance $|t_A - t_B|$ between species A and B, where t_A and t_B are their functional trait values (Kunstler *et al.* 2012). According to Kunstler's method, we defined CWS of all mixtures as the difference of mean pairwise trait distances of species between mixture and

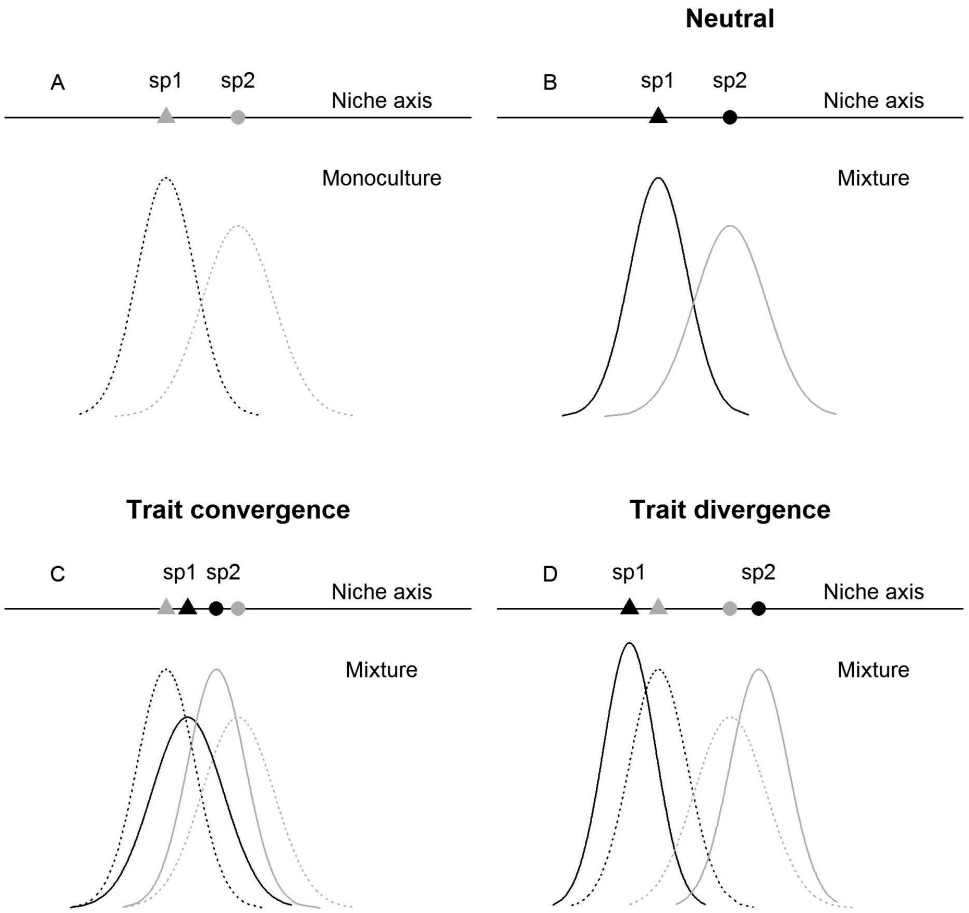

Figure 1: Possible changes in trait values of species grown in monoculture or mixture. Black and grey curves represent trait distributions of species 1 and 2, respectively, while dashed and solid lines represent trait value distributions in monocultures and mixtures, respectively. Triangle and circle indicate mean trait values of species 1 and 2, respectively. Grey and black symbols in the niche axis show mean trait values in monocultures and mixtures, respectively.

monocultures, weighted by the relative abundance of species in mixture (formula 1):

$$\mathrm{CWS} = \sum_{i=1}^{n-1}\sum_{j=2}^{n} P_i P_j \left|T_i - T_j\right|_{mixture} - \sum_{i=1}^{n-1}\sum_{j=2}^{n} P_i P_j \left|T_i - T_j\right|_{monoculture} \quad (1)$$

where P_i, P_j are the relative abundances of species i and j of the total of n species in the mixture under consideration; T_i, T_j are mean trait value of species i and j. CWS represents the variable component of interspecific functional diversity and is the total difference between the absolute trait distance of two randomly selected species in mixture and the absolute trait distance of the corresponding species in monocultures.

Furthermore, if species differ in their trait values between monocultures and mixture, the variable component of intraspecific functional diversity contributes to CE through species interactions. de Bello *et al.* (2013) provide a R function ('trova', i.e. TRaitOVerlAp) to estimate trait dissimilarity between different species, taking into account within-species trait variation. We expanded de Bello's method that had been devised for different species to the case of the same species across different species richness levels and estimated community weighted trait dissimilarity (CWD) from trait dissimilarity between species i in monoculture and mixture ($\mathrm{dissimilarity}_i$), weighted by the relative abundances of species in mixture (formula 2):

$$\mathrm{CWD} = \sum_{i=1}^{n} \mathrm{P}_i * \mathrm{dissimilarity}_i \quad (2)$$

where P_i is the relative abundance of species i and $\mathrm{dissimilarity}_i$ is the trait dissimilarity between species i in monoculture and mixture. Thus, CWD reflects the absolute amount of trait value differences from monoculture to mixture, averaged across all species.

To test for differences in SRL and tree height between different species, separately by species richness levels, we applied analysis of variance (one-way ANOVA), using Tukey's HSD test for *post hoc* comparisons. In addition, we carried out one-way AVOVAs between different richness levels, separately by species. To determine whether over-yielding occurred in mixtures, NE, SE and CE were compared with zero using *t*-tests. Differences in NE, SE and CE between richness levels 2, 4 and 8 were tested with one-way ANOVAs and subsequent Tukey's HSD tests. Finally, we examined the relationships between biodiversity effects (NE, SE and CE) and functional diversity measures (RaoQ, CWS and CWD) by linear regression. We then applied variance partitioning (using the varpart function in the vegan package in R) to assess the relative importance of RaoQ, CWS and CWD in explaining NE and CE. All statistical analyses were done with R 3.0.2 (R Development Core Team 2011).

In the present paper, we do not include abiotic covariates such as topography and soil conditions but focus on the main experimental design factors, i.e. diversity treatments (Schmid *et al.* 2017). Because these treatments were assigned randomly to plots, we had no reason to expect a systematic confounding between diversity treatments and abiotic covariates except to the extent that the diversity treatments themselves might already have changed the abiotic covariates (Schmid *et al.* 2017). In this case, it would not be justified to assign effects to these covariates instead of the diversity treatments. More detailed information about abiotic variables can be found in Kröber *et al.* (2015).

RESULTS

Difference of SRL between species and species richness levels

Five out of the 16 species showed significant plasticity in SRL in response to the four species richness levels (*CaHe*, *TrSe*, *CaSc*, *CyGl* and *KoBi*; Fig. 2) and there was an about 2-fold difference in SRL for these five species across different species richness levels. In the monocultures, SRL was high in *CaHe* and *QuSe* and low in *NySi*, *TrSe*, *CaSc* and *CyGl*. In 2-species mixtures, SRL was high in *CaHe* and *QuSe* and low in *NySi*, *LiFo*, *TrSe*, *ChAx*, *CaSc*, *LiGl* and *KoBi*. In 4-species mixtures, SRL was high in *KoBi* and low in *NySi* and *LiGl*. In 8-species mixtures, SRL was high in *QuFa* and *CaEy* and low in *CaHe*, *NySi*, *TrSe*, *CyGl*, *LiGl* and *KoBi*.

Difference in tree height between species and species richness levels

Ten out of the 16 species showed significant plasticity in tree height in response to the four species richness levels (*CaHe*, *NySi*, *LiFo*, *SaSa*, *TrSe*, *ChAx*, *CaSc*, *QuFa*, *ScSu*, *CyMy*; Fig. 3) and there was an about 1.5-fold difference in tree height for these 10 species across different species richness levels. In the monocultures, tree height was highest in *ChAx* and lowest in *CaEy*. In 2-species mixtures, tree height was highest in *NySi* and lowest in *QuFa*. In 4-species mixtures, tree height was high in *ChAx* and low in *QuSe*, *CaSc*, *CyGl* and *QuFa*. In 8-species mixtures, tree height was high in *ChAx* and low in *QuSe*, *CaSc*, *CyGl*, *QuFa*, *RhCh*, *LiGl*, *KoBi*, *CyMy* and *CaEy*.

Biodiversity effects at different species richness levels

The NE for tree height overall showed a non-significant tendency to increase with species richness but was lowest in 4-species mixtures and highest in 8-species mixtures (Fig. 4A). The CE showed a similar response to species richness as the NE (Fig. 4B). The selection effect (SE) was negative and decreased with increasing species richness (Fig. 4C). Furthermore, when all plots were considered, positive NEs were mainly brought about by positive CEs (Fig. 4D).

Relationships between biodiversity effects and functional diversity measures

The NE of height increased linearly with increasing RaoQ or CWS of SRL, with an amount of explained variance of 18% and 16%, respectively (Fig. 5A and D). The NE marginally increased with CWD (P = 0.09; Fig. 5G). The CE showed

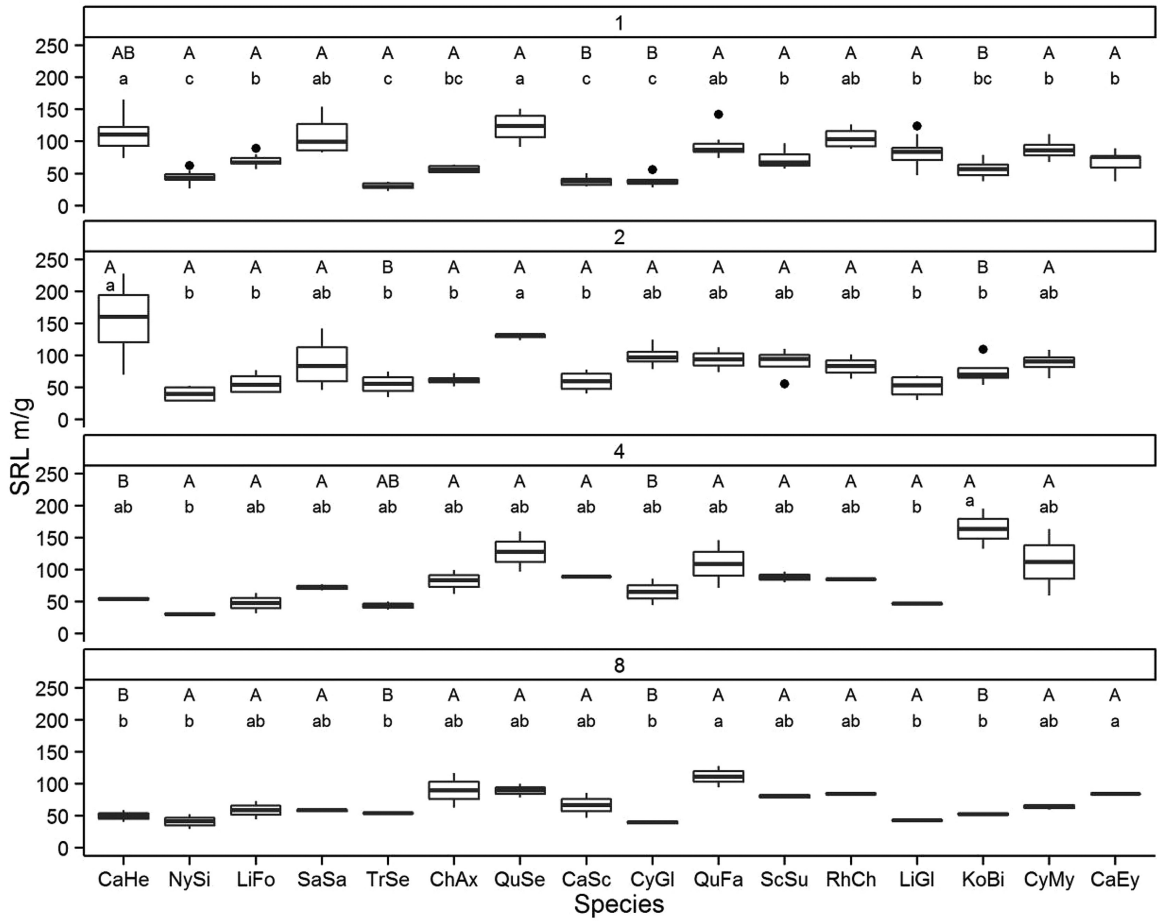

Figure 2: Specific root length (SRL) of all species in the different species richness levels of monoculture, 2-, 4- and 8-spieces mixtures (top to bottom panel). The order of the 16 species along the *x* axis reflects the broken-stick design and shows which species were combined in the different mixtures (cf. Fig. 5A in Bruelheide *et al.*, 2014). Significant differences in SRL between different species richness levels for a particular species are shown in uppercase letters, while significant differences in SRL between different species within species richness levels are shown in lowercase letters ($P < 0.05$).

significant and positive linear relationships with RaoQ, CWS or CWD, with an amount of explained variance of 17%, 21% and 16%, respectively (Fig. 5B, E and H). The SE decreased linearly with increasing CWD (Fig. 5I), but was not significantly related to RaoQ and CWS (Fig. 5C and F).

Results of variance partitioning showed that RaoQ and CWS explained similar variation in NE, with a small amount of variance jointly explained by RaoQ and CWS (Fig. 6A). In contrast, CWS explained more variation than RaoQ and CWD in CE, although there was a large amount of jointly explained variance by RaoQ and CWD (Fig. 6B).

DISCUSSION

Our results demonstrate that species richness can affect SRL and tree height in young experimental stands of subtropical forest species. Although we found a negative NE in 4-species mixtures, brought about by a negative SE and a negative CE, NE and CE showed an overall increasing trend with increasing species richness. Our measures of functional diversity (RaoQ, CWS and CWD) were linearly related to CE, which supports our hypothesis that NE and CE can be explained by RaoQ, CWS and CWD.

Differences in SRL and tree height between species richness levels

The non-significant differences of SRL among species within species richness levels (see Fig. 2) suggest that these species may be subjected to intense competition for niche overlap when they were planted in mixtures (see online supplementary Appendix Fig. A3, negative CWS in 2- and 4-species mixtures). Meanwhile, a significant difference across different species richness levels for 5 of the 16 species (Fig. 2) implies that there is a significant diversity effect on SRL in mixtures, which may be caused by species interactions. Root competition has the potential to decrease the productivity of the competing plants and it may trigger adaptive morphological and physiological responses of the roots and root systems (Hajek *et al.* 2014). However, a non-significant difference across different species richness levels for 11 species demonstrates that

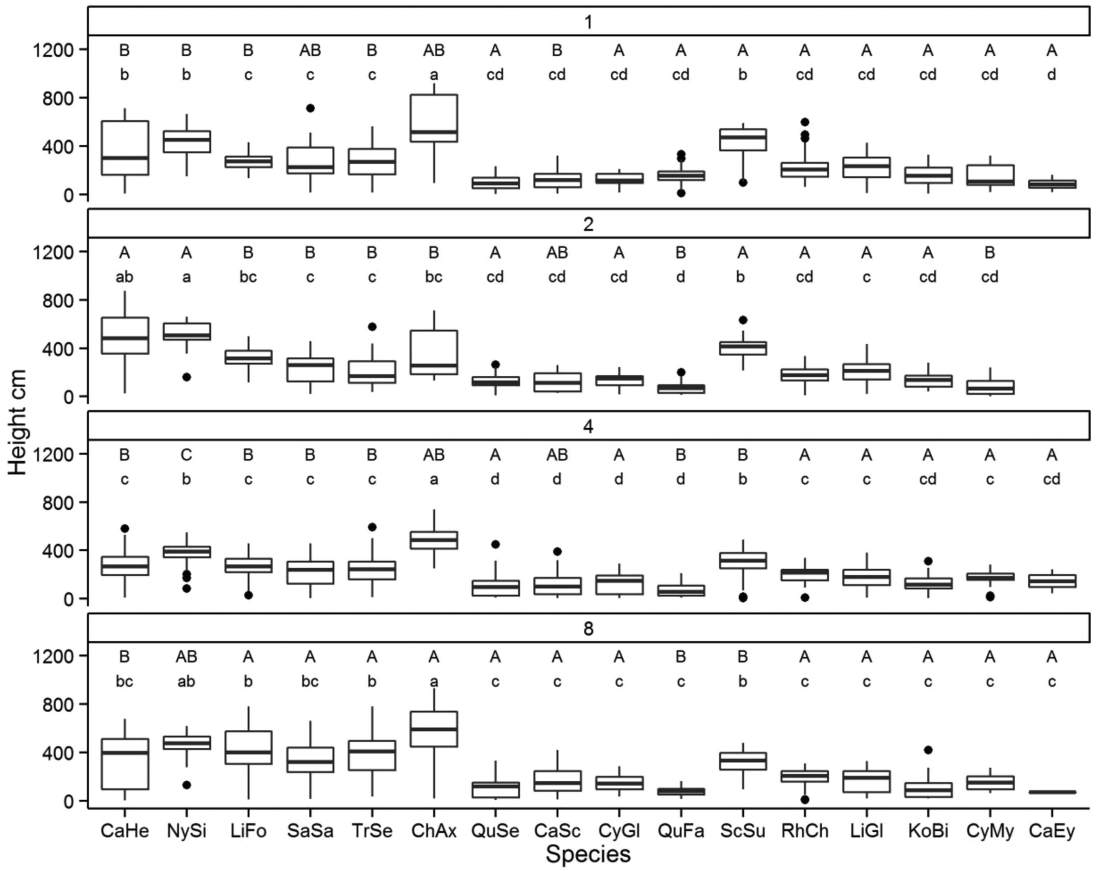

Figure 3: Tree height of all species in the different species richness levels of monoculture, 2-, 4- and 8-spieces mixtures (top to bottom panel). The order of the 16 species along the *x* axis reflects the broken-stick design and shows which species were combined in the different mixtures. Significant differences in tree height between different species richness levels for a particular species are shown in uppercase letters, while significant differences in tree height between different species within species richness levels are shown in lowercase letters ($P < 0.05$).

trait characteristics tend to be similar (niche conservatism) for most of species across different species richness levels (Winter *et al.* 2013). Significant and non-significant differences were also observed for the tree height between species across different species richness levels.

Biodiversity effects at different species richness levels

Overall, both CEs and to a lesser extent SEs played important roles in explaining the observed NEs on tree growth, and CEs showed an increasing trend while SEs declined with increasing species richness (see Fig. 4). A meta-analysis had demonstrated that species mixtures are more productive than the average of all monocultures in 79% out of 44 earlier diversity–productivity experiments (Cardinale *et al.* 2007), and most of the studies showed that both CE and SE positively contributed to NE. In our study, the positive NEs were mainly brought about by positive CEs, because SEs were even negative and decreased with increasing species richness. This suggests that more productive species became more underrepresented in relative abundance in the more species-rich plots. This result is similar to the one from Portugal in the BIODEPTH experiment (Loreau and Hector 2001) and to the result of a study about the relationships between biodiversity and ecosystem functioning in a tropical lowland rainforest (Bu *et al.* 2014). The low values of NE and CE at species richness level four in our experiment indicate that biodiversity effects may not always increase monotonically with species richness. Similar to our study, a close relation between NEs and CEs was also found in the Cedar Creek biodiversity experiment in Minnesota (Cardinale *et al.* 2007; Reich *et al.* 2012), but not in the Jena Experiment in Germany (Roscher *et al.* 2005).

Relationships between biodiversity effects and functional diversity measures

Our results showed that positive NEs were mainly due to positive CEs (see Fig. 4). Recent work has made some progress to analyze the underlying mechanisms for this response pattern (Cardinale *et al.* 2013). The relationships between NE or CE and RaoQ found in our study indicate that the fixed trait differences between species (i.e. trait differences that do not change between monocultures and mixtures) explained 18% of the variation in NEs and 17% of the variation in CEs (see Fig. 5; Botta-Dukát 2005). Since functional traits determine

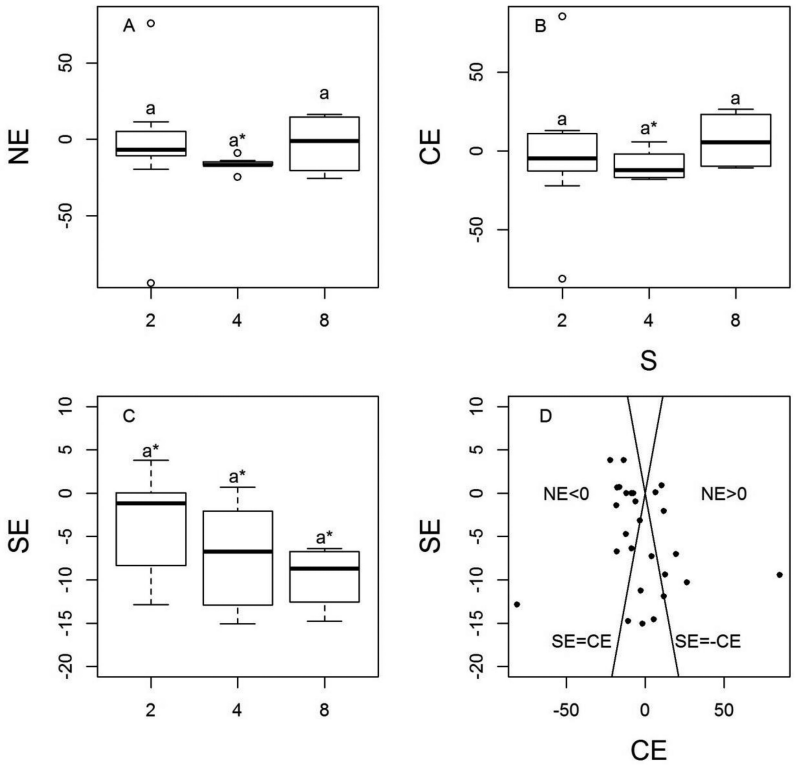

Figure 4: Biodiversity effect across different species richness levels: net effect (NE), complementarity effect (CE) and selection effect (SE). Significant differences between different species richness(s) levels are shown in lowercase letters. Biodiversity effects being significantly different from zero are shown with asterisks ($P < 0.05$).

how an organism extracts resources from its environment (McGill *et al.* 2006), the effects of interspecific trait variation on NEs or CEs suggest that coexistence of species may increase total resource use in a shared soil volume through resource partitioning (Brassard *et al.* 2013). Thus, resource partitioning seems to play an important role in the studied young experimental forest stands.

CWS describes the change in mean niche distances among all species from monocultures to mixture. The linear relationship between CE and CWS suggests that complementarity between species was enhanced by plastic character displacement in mixtures (cf. Zuppinger-Dingley *et al.* 2014). Niche differences between species decrease the strength of interspecific competition relative to intraspecific competition (Chesson 2000). Correspondingly, when the CWS of a community is enlarged with increasing species richness, more complete resource use should promote the performance of the whole community (cf. Brassard *et al.* 2011; Marquard *et al.* 2009; Mommer *et al.* 2010).

The linear relationship between CWD and CE indicates that biodiversity effects were also enhanced by overall trait changes within species from monocultures to mixture. Plants may respond to a competition-induced decrease in resource availability by altering their root morphology to decrease the cost/benefit ratio of resource capturing. Roots may adapt to belowground competition by increasing the specific root surface area and SRL which in turn would reduce the cost of exploiting additional soil volume by the formation of new roots (Hajek *et al.* 2014). Various studies in forest ecosystems observed a morphological root adaptation in response to the presence of competitors (Bolte and Villanueva 2006; Fujii and Kasuya 2008). As a consequence, morphological root adaptations in response to species interaction may contribute to an improved efficiency of capturing resources.

The significant and positive relationships between CWS or CWD and CE suggest that the adjustment in mixture of the root trait considered (SRL) was beneficial to tree growth. The positive relationship between CWS and CE indicates that some species in a mixture exploit easily accessible resources (such as water), thus forcing other species to adjust their SRL to higher or lower values to access less accessible sources. At the same time, the positive relationship between CWD and CE implies that in a mixture the variation of SRL within species becomes also larger, meaning that all species would have high and low SRL to access different water sources at the same time. Therefore, plastic changes in SRL may promote tree growth when species compete for resources such as water and nutrients in mixtures.

Whether mixtures can yield a higher productivity than monocultures may depend on the presence of contrasting functional traits among the component species, which in turn determines the magnitude to which the soil space and its resources are occupied and exploited by roots. However, the key functional trait differences that facilitate or determine

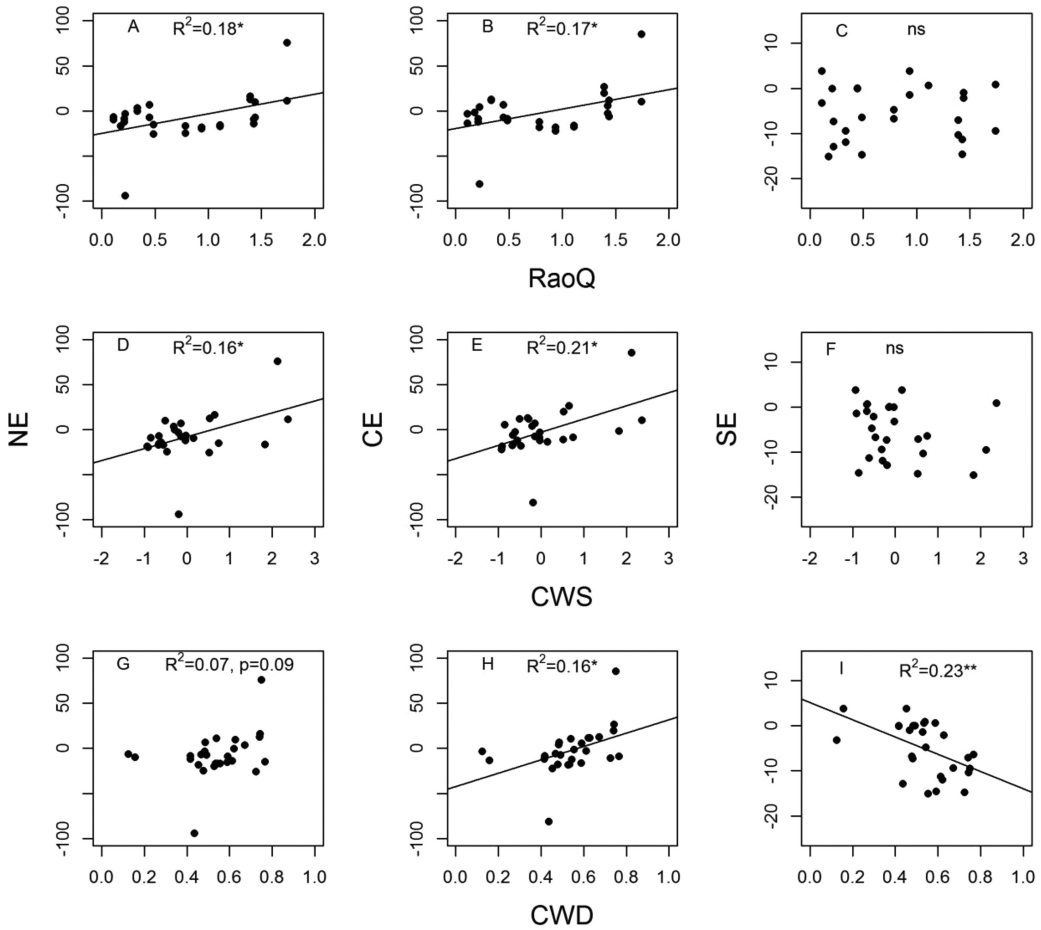

Figure 5: Linear relationships of net biodiversity effect (NE), complementarity effect (CE) and selection effect (SE) with functional diversity (RaoQ), community weighted trait similarity (CWS) and community weighted trait dissimilarity (CWD). Regression lines show significant relationships and R square means the coefficient of determination. *$P < 0.05$, **$P < 0.01$.

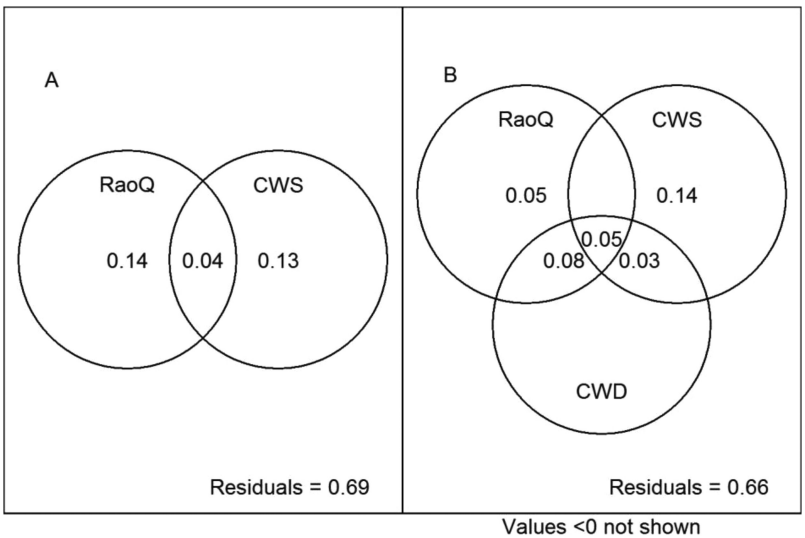

Figure 6: Variance partitioning showing amount of variance in (**A**) net effect (NE) and (**B**) complementarity effect (CE) explained by functional diversity (RaoQ), community weighted trait similarity (CWS) and community weighted trait dissimilarity (CWD).

over-yielding still remain unclear (Brassard *et al.* 2011). We found that CEs were related to RaoQ, CWS and CWD, indicating that complementarity between species depend on resource partitioning and further species interactions. The stronger relationship between CE and CWS than between CE and RaoQ suggests that complementarity was primarily driven by the plastic change of the SRL between coexisting species. This result is consistent with the findings of an 8-year grassland experiment, which showed a novel mechanism for the increase of biodiversity effects: selection for increased niche differentiation through character displacement (Zuppinger-Dingley *et al.* 2014). The large amount of jointly explained variance of CE by RaoQ and CWD means that combining different species in a mixture may also increase the intraspecific variations of species traits. Overall, our results suggest that biodiversity effects in the experimental stands of young subtropical forest trees studied here were driven by both resource partitioning and species interaction and that the variable component of functional diversity (i.e. the plastic changes of species traits from monocultures to mixture) played a more important role for CEs than the fixed component of functional diversity.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology online*.

FUNDING

This project was funded by the National Natural Science Foundation of China (No. 31300353), China Postdoctoral Science Foundation (No. 2014M561089) and Science and Technology Planning Project of Jiangxi Provincial Education Department (No. GJJ150384).

ACKNOWLEDGEMENTS

We are very grateful to Chen Lin and Yang Bo for their contribution to coordinating the fieldwork. We are also grateful to the entire BEF-China research group for their support. The comments of two anonymous reviewers were very helpful to improve the presentation of this paper. BEF-China is funded by the German Research Foundation (DFG FOR891), National Natural Science Foundation of China (NSFC) and the Swiss National Science Foundation (SNSF). We are particularly grateful to the Sino-German Centre for Research Promotion in Beijing. Conflict of interest statement: None declared.

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